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ORIGINAL ARTICLE

Antioxidant system status of cucumber plants under pesticides treatment

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Abstract

A plants' physiology maybe affected by various pesticides through the activation or inactivation of different biochemical pathways in target and non-target plants. In response to pesticides as xenobiotics, plants activate their antioxidant defense systems through both enzymatic and non-enzymatic pathways. In this study, two of the most common pesticides used to control cucumber whiteflies, imidacloprid and dichlorvos were sprayed on cucumber seedlings. Treatment with both pesticides significantly increased the activity of superoxide dismutase, catalase, ascorbate peroxidase, guaiacol peroxide, and phenylalanine ammonia-lyase. Moreover, total protein, proline, total soluble carbohydrates, and total phenolic content showed a significant elevation in response to the treatment with both pesticides compared to the control. The effects of the separate use of pesticides resulted in variation in the peak day of physiological changes in treated plants. Further experiments showed that pesticide treatment leads to a significant decrease in polyphenol oxidase activity, but no significant changes in contents of hydrogen peroxide, malondialdehyde, and electrolyte leakage index were found. Our results suggest that imidacloprid and dichlorvos had profound effects on the physiological status of cucumber plants at recommended rates. Our data also showed that the responses were similar between the two pesticides with differences in response times following treatment.

Keywords Antioxidative system · Cucumber · Pesticides · Oxidative stress · Plant physiology

Introduction

Plants experience various types of stress during their growth and development, which cause different types of biological responses (Ahmad et al. 2017). Through evolutionary processes, plants have developed a wide range of defense mechanisms to protect themselves from various reactive oxygen species (ROS), which are highly reactive molecules (Singh et al. 2019). The antioxidant system of plants consists of both enzymatic and non-enzymatic antioxidants, which helps plants to survive in a stressful condition (Ahmad et al. 2017). The metalloenzyme superoxide dismutase (SOD) by dismutation of O_2^- to O_2 and hydrogen peroxide (H_2O_2) molecules forms the first line of plant defense system against oxidative stress. Catalase (CAT) catalyzes the dismutation of two H_2O_2 molecules to H_2O and O_2 (Sharma et al. 2012). Ascorbate peroxidase (APX), one of the central components of the ascorbate–glutathione cycle with a significant affinity for H_2O_2, is responsible for the degradation of H_2O_2 using two molecules of ascorbic acid as a reductant. Guaiacol peroxidase (GPX) removes H_2O_2 by oxidizing aromatic electron donors like guaiacol and pyrogallol (Gill and Tuteja 2010). Phenylalanine ammonia-lyase (PAL) is a key enzyme in plant secondary metabolism, which synthesizes plants phenolic compounds (Sharma et al. 2019). Polyphenol oxidase (PPO) catalyzes the reaction in which the oxidation of the phenols produces *o*-quinones, the highly reactive molecules that make cross-links with plant proteins (Boeckx et al. 2015). Plant proteins, which in most cases play enzymatic roles, are another critical part of the chemical machinery for plant growth and development (Day 1996). Plants also accumulate proline as an organic osmolyte in stressful

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conditions to improve their tolerance and it has been shown that proline also plays a role in scavenging ROS (Kaur and Asthir 2015). In addition, the soluble carbohydrates of plants play an actual role in responses to stress conditions that is called sweet immunity in plants (Bolouri Moghaddam and Van den Ende 2013). Phenolic compounds are important secondary metabolites of plants, which have antioxidant properties to quench ROS (Kubalt 2016). H_2O_2 is a well-recognized signaling molecule due to its diffusibility and relatively long-lived features. H_2O_2 has an influential role in plant defense systems, the conducive content of which is normally maintained in a dedicating balance between the production and scavenging (Černý et al. 2018). Additionally, malondialdehyde (MDA) is an indicator of membrane lipid peroxidation and electrolyte leakage index (ELI), as injury indices, are important components of a plant's immune system which are used to assess plant health status in responses to (a)biotic stresses (Heidarvand and Maali-Amiri 2013). Plants equipped with strong antioxidant capacities are better equipped to overcome stressors.

On the other hand, it is a well-documented fact that often less than 0.1% of applied pesticides actually reach the pests of crops (Pimentel 1995). Therefore, the effects of pesticides are not limited to target organisms but on a wide range of non-target organisms, like plants themselves (Szczepaniec and Raupp 2013). Regarding the chemical characteristics of pesticides and biochemical composition of plants, it is expected that pesticides interact and may interfere with different biochemical pathways of plants (Shakir et al. 2018). Based on previous research, plant physiological and biochemical pathways are obviously influenced by pesticides as stressful chemical compounds, but little is known about the effect of pesticides on antioxidant dependent defense pathways in plants. In addition to this, many questions are still unanswered for physiological responses of plants under pesticides pressure. Thus, in the last 10 years, many studies have focused on plant-pesticide relationships to shed light on the events involved in the interactions between plants chemical defense systems and common pesticides.

The present study focuses on the physiological interactions of two different pesticides and cucumber plants, which are hosts for the insects controlled by these pesticides. Cucumber (*Cucumis sativus* L.) is one of the most commonly cultivated vegetable crop in the world (Kaewkham et al. 2016). Furthermore, a number of herbivorous insects attack cucumber, including the tobacco whitefly (*Bemisia tabaci*) (Liang et al. 2012). During the last several decades, numerous pesticides from various chemical groups have been used to control this pest including imidacloprid and dichlorvos (Liang et al. 2007). Imidacloprid, a systemic pesticide, is a neonicotinoid that affects an insect's nervous system (Wang et al. 2002) and dichlorvos, which is an organophosphorus contact pesticide, acts as the acetylcholinesterase inhibitor of the insect's nervous system (Wang et al. 2004).

The objective of the present study was to investigate how a plant's chemical defense system responds to pesticides including two insecticides without an understood site of action in plants for the first time. To address this objective, the antioxidant system of cucumber plants was evaluated after exposure to the pesticides imidacloprid and dichlorvos. The results of this study may shed light on integrating pesticide applications with the plant physiological cycle to maximize pest control. Therefore, networks of metabolic pathways that determine the physiological status of cucumber plants could be targeted for future pesticide synthesis and their application in integrated pest management programs.

Materials and methods

Plants and pesticides

Pure and uniform seeds of hybrid super N3 cucumber cultivar obtained from HED company, USA, were planted and grown in 15 cm-diameter plastic pots of sterilized soil composed of 1:1:2 cocopeat:peat moss:perlite. Plants were contained in a greenhouse under controlled conditions of 16/8 h light/dark photoperiod, light intensity 5100 lx, the temperature of 26 ± 2 °C and 30–40% relative humidity. Plants were watered every 3 days. After reaching the intended phenological stages (6–8 true leaves), recommended rates of imidacloprid, 0.14 g a.i./l, (Confodir® SC 350, Bayer CropScience, Germany) and dichlorvos, 0.4 g a.i./l, (Dichlorvos® EC 50%, Ariashimi Company, Iran) for control of the tobacco whitefly in the greenhouse were applied to 30-day-old cucumber seedlings. Simultaneously, control plants were treated with deionized water (DI water). After pesticides were applied, whole cucumber leaves were collected at regular intervals of 0, 2, 4, 6, 8, 10, 12, and 14 days after treatment (DAT) based on pre-tests results. The 0 DAT samples were taking after spraying, when no traces of pesticides remaining on leaves. All collected leaf tissues were freeze dried and then ground in liquid nitrogen and stored at − 80 °C until further use.

Enzymatic and non-enzymatic parameters assessment

To determine the effect of pesticide treatments on cucumber plants, several enzymes including SOD, CAT, APX, GPX, PAL, PPO, and non-enzymatic parameters including the contents of total protein, proline, total soluble carbohydrates, total phenolic compounds, H_2O_2, MDA, and ELI were investigated in pesticide-treated and control leaf samples.

The activity of SOD and CAT were analyzed based on the methods described by Acar et al. (2001) and Aebi (1984), respectively. SOD activity was calculated based on the inhibition in the photochemical reduction of nitroblue tetrazolium (NBT). Briefly, after homogenization of leaf tissue in 50 mM phosphate buffer (pH 7.8), 66 mM ethylenediaminetetraacetic acid (EDTA), 10 mM methionine, 33 µM NBT, and 33 µM riboflavin were added to the mixture before incubation at 25 °C for 20 min under a fluorescent light and dark condition. The absorbance of the total solution was measured at λ = 560 nm. To determine CAT activity of plant samples, leaf tissue was homogenized in 50 mM phosphate buffer (pH 7). The decrease in absorbance value of reaction mixtures comprised of a sample extract and 30 mM H_2O_2 as a substrate was considered as the method principle. Absorbance values were recorded at λ = 240 nm (ε = 0.000394 mM^{-1} cm^{-1}).

According to the method of Madhusudhan et al. (2003), APX activity of leaf sample was measured by adding 50 mM phosphate buffer (pH 7) to the sample. The absorbance value of reaction mixtures including 1 mM EDTA, 0.1 mM H_2O_2 as a substrate, 0.4 mM ascorbic acid as a reductant was recorded at λ = 290 nm (ε = 2.8 mM^{-1} cm^{-1}). For GPX activity measurement leaf samples, the absorbance value of a reaction mixture containing 5 mM guaiacol as an electron donor, 15 mM H_2O_2 as a substrate, 0.1 µM EDTA, added to the sample was measured at λ = 470 nm (ε = 26.6 mM^{-1} cm^{-1}) as described by de Azedevo Neto et al. (2006).

PAL activity was determined based on the rate of cinnamic acid (CA) production as described by Wang et al. (2006). To do this, leaf tissue was homogenized in 50 mM Tris–HCl buffer (pH 8.8). Then, 10 mM phenylalanine (as substrate) was added and the mixture was incubated at 37 °C for 1 h. Afterwards, the reaction was stopped with 6 M HCl and produced CA was extracted by ethyl acetate. After suspending the solution in 0.05 M NaOH, CA concentration was quantified by the absorbance value measurement at λ = 290 nm. PAL activity was assayed by the extinction coefficient equal to 9500 M^{-1} cm^{-1}. PPO activity was evaluated based on Ögel et al. (2006). Enzyme extraction from plant tissue was carried out in 0.1 M phosphate buffer (pH 7). Then, the extract was measured, including an addition of 0.1 M catechol (as substrate) at λ = 420 nm. PPO activity was assayed according to increased absorbance of *o*-quinone produced with an extinction coefficient 3450 mM^{-1} cm^{-1}.

For the physiological parameters classified as non-enzymatic plant characteristics, total protein, proline, total soluble carbohydrates, total phenols, H_2O2, MDA, and ELI were assessed. Total protein content in treated and control plants was measured by the method of Bradford (1976) using Coomassie Brilliant Blue G-250 and absorbance was recorded at λ = 595 nm with serial dilutions of the bovine serum albumin as the standards.

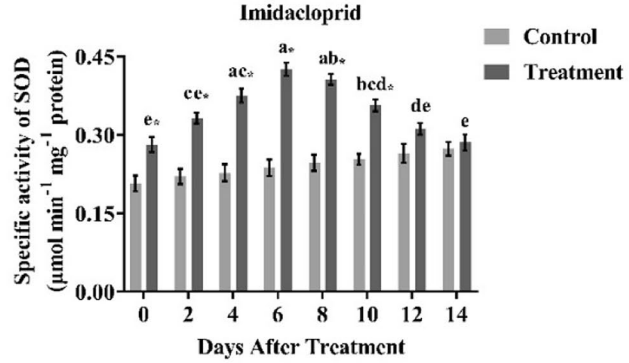
Proline content of samples was determined by the ninhydrin-based colorimetric assay asdescribed by Carillo and Gibon (2011) in which the cold extraction procedure with 40% (v/v) ethanol was used to extract proline from leaf samples. Ninhydrin 1% (w/v) in acetic acid 60% (v/v) and ethanol 20% (v/v) was used as the reagent. The absorbance of the reaction mixtures was read at λ = 520 nm. To determine the content of total soluble carbohydrates, the method described by Laurentin and Edwards (2003) was followed. Extraction from leaf tissue was accomplished using DI water. Anthrone 0.2% (w/v) in sulfuric acid 98% was used as the reagent. In addition, a glucose solution in DI water was used as standard. Finally, the sample absorbance was recorded at λ = 620 nm.

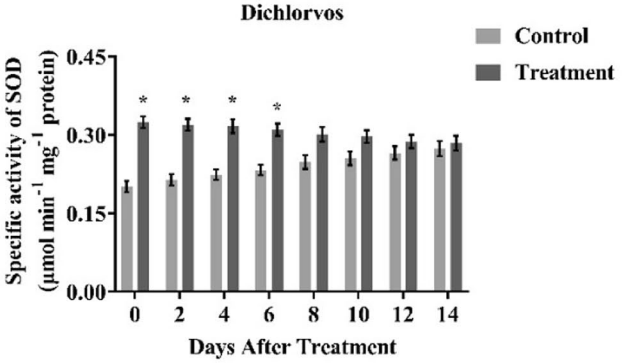
Total phenolic content of leaves was measured in leaf tissue using 95% methanol as described by Ainsworth and Gillespie (2007). After sample preparation in methanol, Folin–Ciocalteu reagent was used in an alkaline medium (700 mM sodium carbonate) to form blue complexes needed for determination of phenolic compounds at λ = 760 nm. Gallic acid solution in 95% methanol were utilized as the standard. H_2O_2 content of collected tissues was estimated according to the method of Velikova et al. (2000), which is based on potassium iodide (KI) oxidation by H_2O_2 in an acidic medium. H_2O_2 was extracted from leaf tissue using 0.1% (w/v) trichloroacetic acid (TCA). The absorbance value of reaction mixture including 10 mM phosphate buffer (pH 7), 1 M KI and extract solutions was recorded at λ = 390 nm. Solutions of H_2O_2 in 0.1% (w/v) TCA were used as the standards.

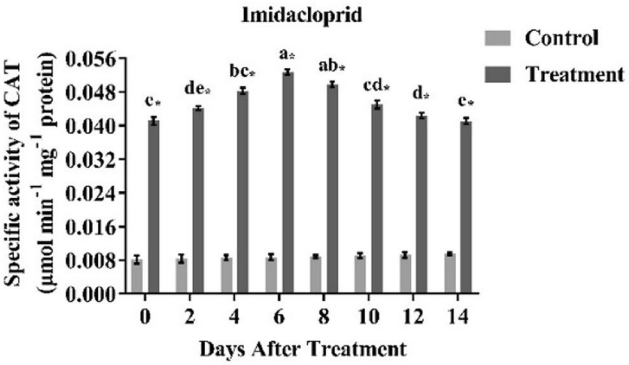
MDA content was quantified using the thiobarbituric acid test as stated by Sunkar et al. (2006). Measurement was performed at λ = 532 nm and λ = 600 nm and MDA content was calculated with extinction coefficient 155 mM^{-1} cm^{-1}. ELI was analyzed based on the method described by Murray et al. (1989). After treatment, each leaf was placed in a polypropylene vial to which was added DI water. The conductivity of the solution was measured using a platinum electrode with a thermistor for automatic temperature correction. The conductivities were measured every 3 h during the first day and daily thereafter. The samples were stored in the dark at 4 °C between measurements. At the end of 1 week, samples were autoclaved at 105 °C for 4 min. A total conductivity value was obtained by measuring the conductivity of the autoclaved solution.

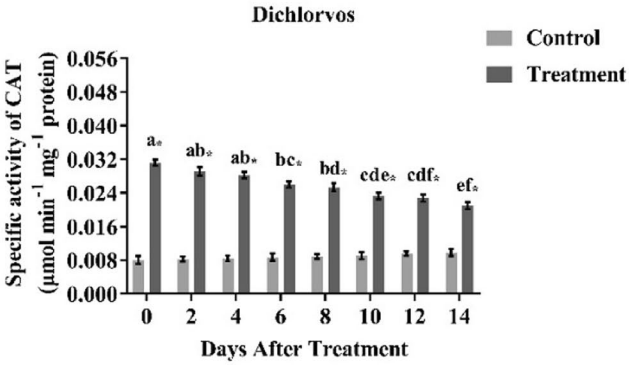
Statistical analysis

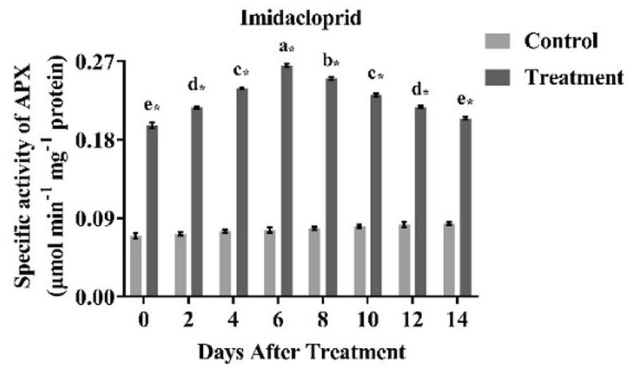
Data were analyzed with analysis of variance (ANOVA) for each variable. Treatments in each plant were consigned to a randomized complete block design with three independent

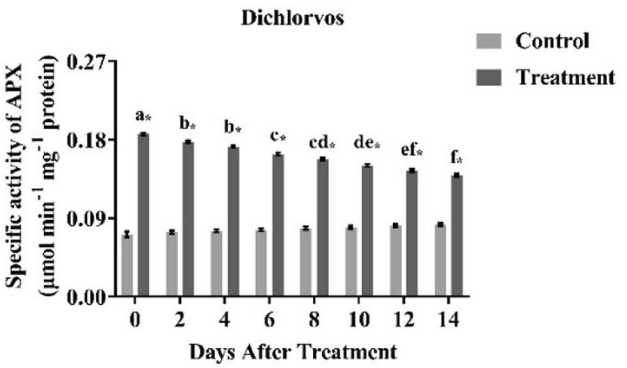

Imidacloprid
Control
Treatment
Specific activity of SOD (μmol min-1 mg-1 protein)
0.45
0.30
0.15
0.00
e* ce* ac* a* ab* bcd* de e
0 2 4 6 8 10 12 14
Days After Treatment

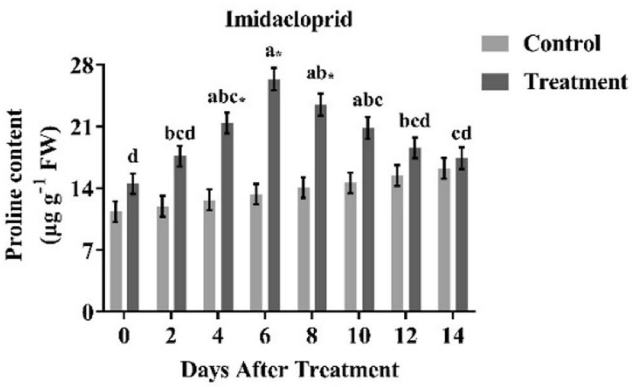

Dichlorvos
Control
Treatment
Specific activity of SOD (μmol min-1 mg-1 protein)
0.45
0.30
0.15
0.00
* * * *
0 2 4 6 8 10 12 14
Days After Treatment

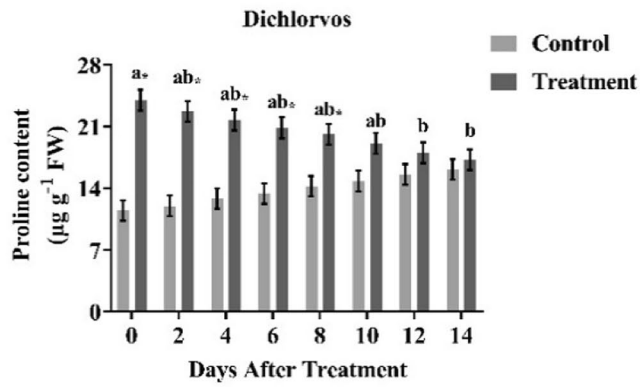

Imidacloprid
Control
Treatment
Specific activity of CAT (μmol min-1 mg-1 protein)
0.056
0.048
0.040
0.032
0.024
0.016
0.008
0.000
c* de* bc* a* ab* cd* d* c*
0 2 4 6 8 10 12 14
Days After Treatment


Dichlorvos
Control
Treatment
Specific activity of CAT (μmol min-1 mg-1 protein)
0.056
0.048
0.040
0.032
0.024
0.016
0.008
0.000
a* ab* ab* bc* bd* cde* cdf* ef*
0 2 4 6 8 10 12 14
Days After Treatment


Imidacloprid
Control
Treatment
Specific activity of APX (μmol min-1 mg-1 protein)
0.27
0.18
0.09
0.00
e* d* c* a* b* c* d* e*
0 2 4 6 8 10 12 14
Days After Treatment


Dichlorvos
Control
Treatment
Specific activity of APX (μmol min-1 mg-1 protein)
0.27
0.18
0.09
0.00
a* b* b* c* cd* de* ef* f*
0 2 4 6 8 10 12 14
Days After Treatment


Imidacloprid
Control
Treatment
Proline content (μg g-1 FW)
28
21
14
7
0
d bcd abc* a* ab* abc bcd cd
0 2 4 6 8 10 12 14
Days After Treatment


Dichlorvos
Control
Treatment
Proline content (μg g-1 FW)
28
21
14
7
0
a* ab* ab* ab* ab* ab b b
0 2 4 6 8 10 12 14
Days After Treatment

◂**Fig. 1** Superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX) specific activity (μmol min^{-1} mg^{-1} protein) during 14 DAT in cucumber seedlings in response to imidacloprid and dichlorvos. Different letters indicate significant differences among different days in treated plants and asterisks were used to show statistically significant difference between treated and control plants in each DAT

biological replicates per treatment. A two-way ANOVA was used to compare treated plants on different days and also differences between the non-treated and treated plants at each day of harvest. The trait means were compared with Tukey test at 0.05 probability level. All analyses were performed in GraphPad Prism version 8.2.0 (La Jolla California, USA) which also was used to draw charts.

All spectrophotometric experiments were carried out using the UV/VIS spectrophotometer (Optizen POP™, South Korea).

Results

Enzymatic parameters

Based on enzymatic activity assays, the specific activity of SOD in imidacloprid-treated plants showed a significant increase at 0 till 10 DAT compared to the control. A similar upward trend in SOD activity was recorded for plants exposed to dichlorvos at 0 till 6 DAT. In addition, the highest amount of SOD was reported at 6 DAT in imidacloprid-treated plants with 1.79-fold higher SOD levels compared to SOD in control plants. There was no singular maximum activity DAT for plants exposed to dichlorvos (Fig. 1).

The specific activities of CAT, APX, and GPX in imidacloprid-treatment plants significantly increased during the whole experimental period and reached a maximum level at 6 DAT with 6.05, 3.46, and 3.82 fold higher enzyme activities compared to control plants, respectively (Fig. 1). Similarly, the dichlorvos-treated plants showed significantly higher CAT, APX, and GPX specific activity during the whole experimental period, with maximum activity at 0 DAT with 3.87-, 2.60-, and 2.30-fold higher enzyme activity, respectively (Fig. 1).

Experiments also revealed that the specific activity of PAL in imidacloprid-treated plants reached peak activity at 6 DAT (1.60-fold). A significant increase occurred at 0 till 12 DAT compared to the control. Treatment with dichlorvos was similar to imidacloprid whereby significantly higher PAL specific activity in plants occurred at 0 till 6 DAT compared to the control; however, no remarkable maximum activity was observed (Fig. 2).

In a sharp contrast with the rest of the enzymes analysed, the specific activity of PPO in imidacloprid-treated plants during the whole experimental period was significantly lower compared to the control, with the lowest value observed 6 DAT (1.88-fold decrease in activity). Similarly, the dichlorvos-treated plants showed significantly lower PPO specific activity compared with the control at 0 till 12 DAT. The lowest PPO activity in pesticide-treated plants was reported at 0 DAT with 1.32 times decrease compared to the control (Fig. 2).

Non-enzymatic parameters

The total protein content of plants significantly increased compared with the control in both imidacloprid and dichlorvos-treated plants, which occurred from 2 till 12 DAT for imidacloprid and 0 till 8 DAT for dichlorvos treatments. The peak days of total protein levels in imidacloprid- and dichlorvos-treated plants occurred at 6 and 0 DAT (with 1.89- and 2.08-fold more total protein content), respectively (Fig. 3).

As shown in Fig. 3, the total soluble carbohydrate content in imidacloprid-treated plants showed a significantly increasing trend compared to the control at 2 till 14 DAT. Also, the soluble carbohydrate content in response to dichlorvos significantly increased at 0 till 8 DAT. The highest content of the total soluble carbohydrates for imidacloprid-exposed plants was recorded at 6 DAT. However, dichlorvos-treated plants showed no meaningful peak day.

Imidacloprid-treated plants exhibited a significantly increasing trend for total phenol content compared to the control at 2 till 10 DAT and the highest level was at 6 DAT with 1.84-fold higher than the control plants. Compared to the control, there was also a significant increase in total phenol content of dichlorvos-treated plants at 0 till 12 DAT, with the maximum level at 0 DAT with 1.96-fold higher than the control plants (Fig. 3).

The highest level of proline in plants exposed to imidacloprid, occurred at 6 DAT (1.97-fold). Higher concentrations of proline were observed at 4 till 8 DAT compared to the control. Also, the proline content in response to dichlorvos treatment showed a significant increase at 0 till 8 DAT. The highest content of proline was at 0 DAT with 2.08-fold more than in the control plants (Fig. 3).

Surprisingly, H_2O_2 and MDA content in addition to ELI did not show any significant change in imidacloprid and dichlorvos-treated plants compared to the control plants (Fig. 4).

Discussion

The overall physiology of plants is affected by xenobiotics like pesticides in different ways (Jones et al. 1986). In this study, cucumber plant responses were recognized in the plant defense system, which induced by these pesticides. Detailed results revealed that treatment with imidacloprid

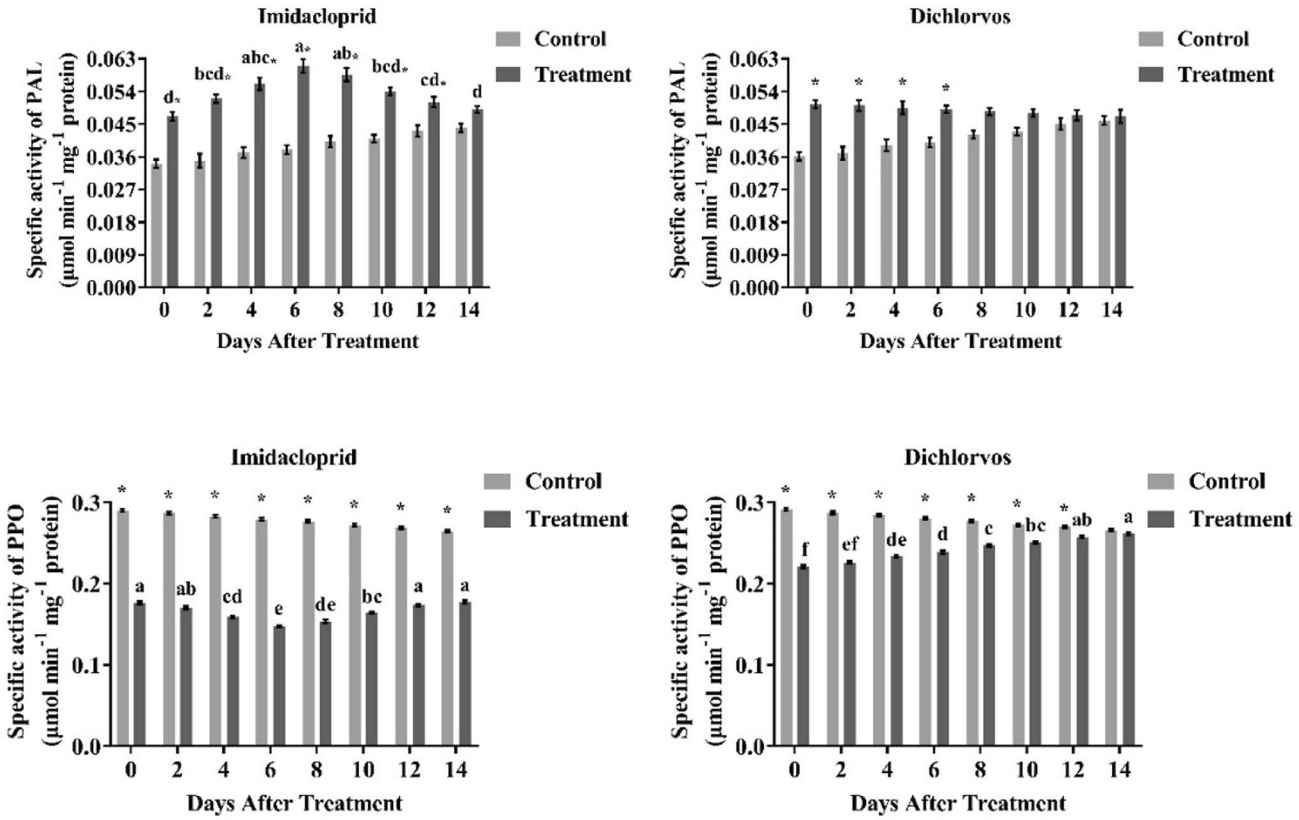


Fig. 2 Phenylalanine ammonia-lyase (PAL) and polyphenol oxidase (PPO) specific activity (µmol min^{-1} mg^{-1} protein) during 14 DAT in cucumber seedlings in response to imidacloprid and dichlorvos. Different letters indicate significant differences among different days in treated plants and asterisks were used to show statistically significant difference between treated and control plants in each DAT

and dichlorvos significantly increased SOD, CAT, APX, GPX and PAL enzyme activity. Also, total protein, proline, total soluble carbohydrates, and total phenol content significantly increased in plants exposed to pesticides. However, pesticides had no significant effect on the contents of H_2O_2, MDA, and ELI and actually decreased the activity of PPO.

Consistent with previous studies, each plant exposed to abiotic stresses accumulates ROS, which causes oxidative damage to macromolecules and metabolites in the cell (Noctor and Foyer 1998). Then to protect plants from the harmful effects of oxidative stresses, enzymatic and non-enzymatic antioxidant become active and stored in high content to scavenge ROS (Foyer and Shigeoka 2011). From this perspective, the results of the present study exhibit profound physiological and biochemical changes of cucumber plants in response to these pesticides.

Every enzymatic and non-enzymatic antioxidant play unique roles and stimulate or potentate each other to take part in the plant defense system. SOD acts as the catalyzer in a dismutation reaction which converts the superoxide radicals into molecular oxygen and H_2O_2 (Alscher et al. 2002). H_2O_2 acts as a signaling molecule in the plant immune system at low concentrations; however, it has the ability to cause programmed cell death at higher concentrations (Neill et al. 2002). By contrast, CAT, APX (Černý et al. 2018), and GPX (Noctor and Foyer 1998) that can be stimulated by H_2O_2, take part in the H_2O_2 scavenging process. The reported increase of activity of SOD in cucumber seedlings might be a reason for H_2O_2 production increase. However, stability in the H_2O_2 levels can be related to a significant increase in CAT, APX, and GPX activities. On the other hand, non-changes in ELI and MDA content, as injury indices, clearly showed that neither imidacloprid nor dichlorvos is not a stressing agent for cucumber plants and H_2O_2 act as a signaling molecule to induce plant's antioxidant system.

Soluble carbohydrates in low contents act as a signaling molecules in the induction of the plant defense system and can directly attack the ROS when in high concentrations (Van den Ende 2014). Soluble carbohydrates can cause an increase SOD activity by modification in its gene expression (Keunen et al. 2013). On the other hand, soluble carbohydrates can increase PAL activity as well (Camm and Towers 1973), which leads to an increased synthesis of phenolic compounds through the phenylpropanoid pathway. From this point of view, the increased level of total soluble carbohydrate content, SOD and PAL activity, and total phenol

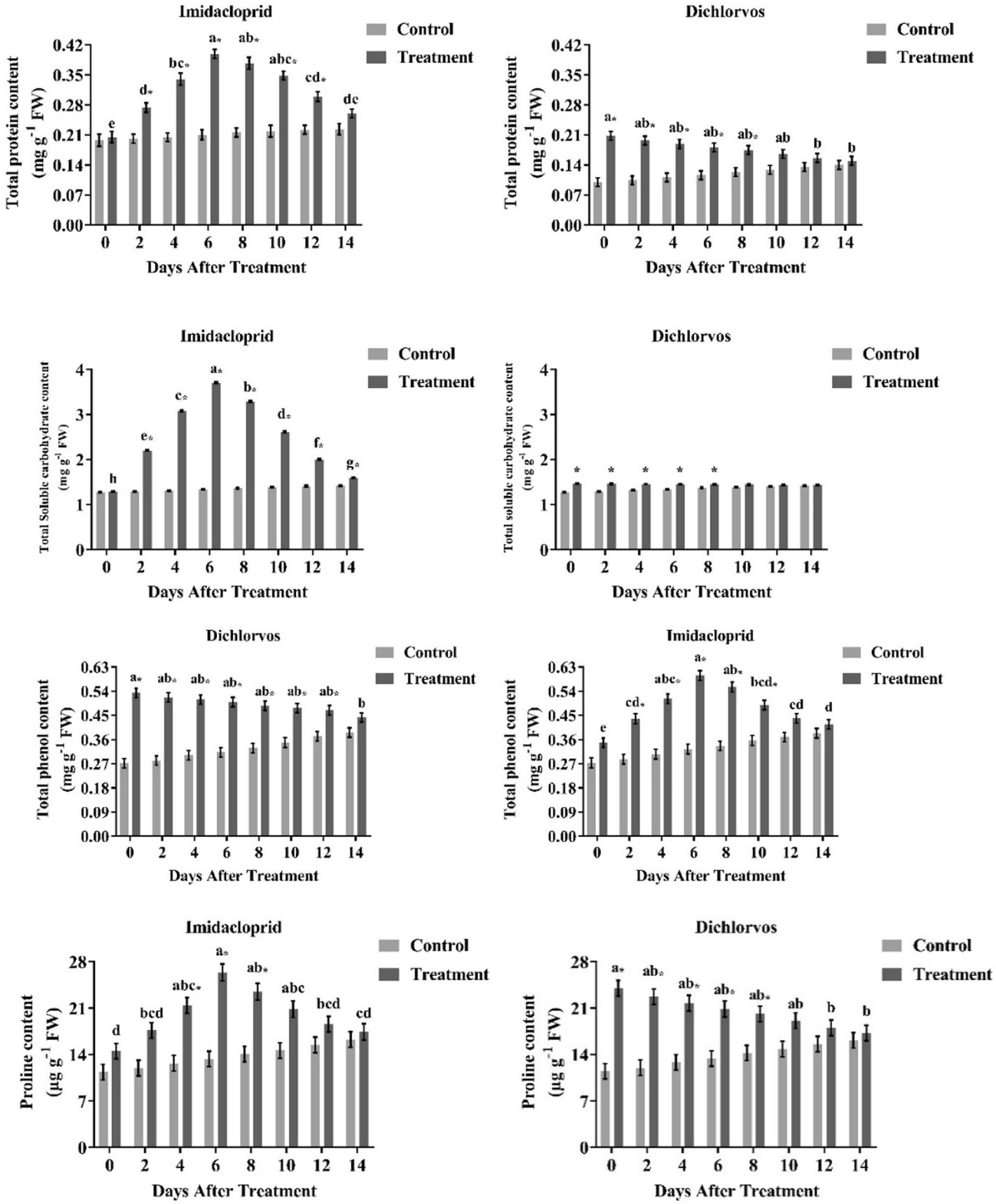


Fig. 3 Content of total protein, total soluble carbohydrate, total phenol (mg g^{-1} FW), and proline (µg g^{-1} FW) during 14 DAT in cucumber seedlings in response to imidacloprid and dichlorvos. Different alphabet indicates significant differences among different days in treated plants and asterisks were used to show statistically significant difference between treated and control plants in each day

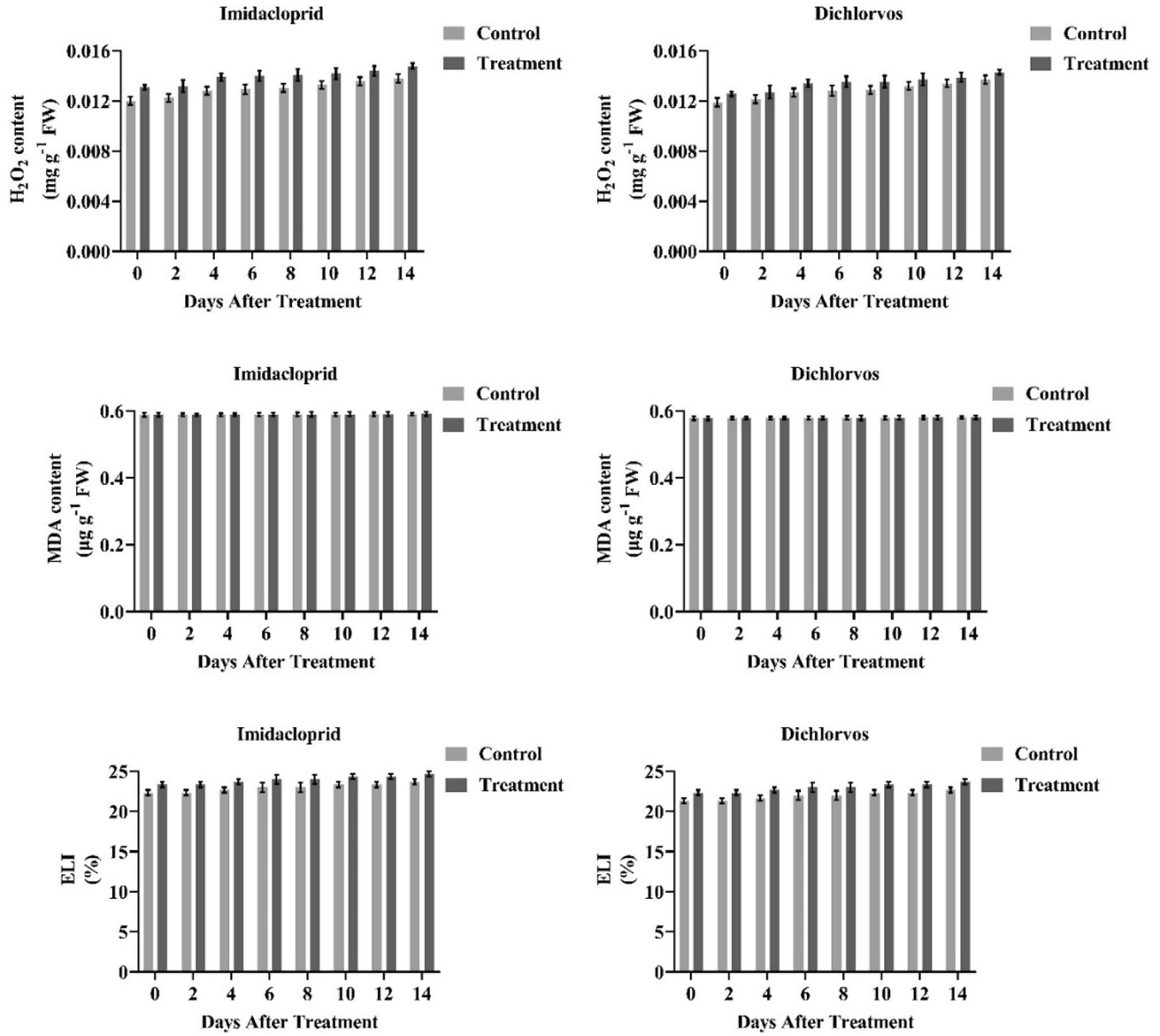


Fig. 4 Content of hydrogen peroxide (H_2O_2) (mg g^{-1} FW), malondialdehyde (MDA) (µg g^{-1} FW), and electrolyte leakage index (ELI) (%) during 14 DAT in cucumber seedlings in response to imidacloprid and dichlorvos

contents in pesticide-treated plants shows the interrelation of these components with each other.

Proline accumulates in plants as an osmolyte in response to (a)biotic stresses (Hayat et al. 2012) and its biosynthesis provides NADPH to activates the ascorbate–glutathione cycle. Consequently, proline biosynthesis can also activate APX. Meanwhile, soluble carbohydrates regulate proline synthesis and cause proline accumulation (Kaur and Asthir 2015). On the other hand, proline content increase leads to an elevated PAL (Silva et al. 2018), CAT, and SOD activity (Kaur and Asthir 2015) as well as increased phenolic compound productions (Silva et al. 2018). Protein integrity protection, prevention of protein aggregation and protein stabilization are other functions of proline (Fedotova 2019). Therefore, the rise in proline content of plants treated with imidacloprid and dichlorvos can be associated with the activation of enzyme activities and the increase of the other non-enzymatic parameter.

As reported before, a decrease in PPO activity indicates decreases of *o*-quinone production, which produces cross-links with proteins (Boeckx et al. 2015). Phenolic compounds are PPO inhibitors that inhibit activity by chelating copper in the PPO structure (Mayer 2006). This phenomenon results in protein quantity and quality improvement (Kroll and Rawel 2001). It is well reported that the increased protein content is linked with increased activity of enzymatic

and non-enzymatic defense related proteins produced during stress (War et al. 2015). In line with previous studies, the recorded decrease in PPO activity in pesticide-treated cucumber plants in this study maybe related to elevated levels of phenolic compounds in these plants. Moreover, our results have shown that there is a relationship between the increase of protein content in plants exposed to imidacloprid and dichlorvos pesticides, the increase of enzymes activities and even the PPO activity decrease of plants.

No significant change in content of MDA, maybe accounted by increased content of phenols (Sharma et al. 2012) and proline (Czarnocka and Karpiński 2018) because these compounds have an ability to inhibit lipid peroxidation and result in the consistency of MDA content.

Physicochemical features are the most important characteristics of pesticides, which determine a plants response to various pesticides. For instance, different leaf penetration rates of various pesticides maybe due to differences in physicochemical properties (Lichiheb et al. 2016). Vapor pressure (VP), Henry's law constant (HLC), solubility, and octanol/water partition coefficient (Kow) of pesticides are the most important physicochemical properties that affect the response of plants to pesticides (Linde 1994; Zacharia 2011).

VP which is a property of a liquid-based material on the strength of its intermolecular forces (Linde 1994; Zacharia 2011), at 25 °C for dichlorvos and imidacloprid is equal to 2.1×10^{3} mPa and 9×10^{-7} mPa, respectively (MacBean 2018). These properties indicate that dichlorvos evaporates more easily than imidacloprid. Thus, dichlorvos has less time to penetrate into the plant cuticle compared to imidacloprid. HLC is the proportion of a material's chemical concentration in the air over its concentration in water (Linde 1994; Zacharia 2011). HLC is recorded 2.58×10^{-2} Pa m^3 mol^{-1} and 1.7×10^{-10} Pa m^3 mol^{-1} for dichlorvos and imidacloprid (at 20 °C), respectively (MacBean 2018), which means that dichlorvos has more of a tendency to evaporate from aqueous solutions compared with imidacloprid. Based on this, the persistence of imidacloprid on the sprayed surface of plants is higher than that for dichlorvos, and thus the imidacloprid has more time to penetrate the plant leaf cuticle. The solubility of a pesticide determines the dissolvability of it in a polar solvent (Linde 1994; Zacharia 2011) which is about 16.4 g/l and 0.61 g/l at 20 °C, for dichlorvos and imidacloprid, respectively (MacBean 2018). It is clear that the dichlorvos, with higher water solubility, cannot penetrate the hydrophobic layers of plant cuticle as readily as imidacloprid. Kow is the ratio of a material's chemical concentration in octanol over its concentration in water (Linde 1994; Zacharia 2011). Based on the fact sheets, the Kow of dichlorvos and imidacloprid is 1.9 and 0.57 (at 20 °C and pH 7), respectively (MacBean 2018). Thus, imidacloprid can penetrate throughout the plant cuticle lipophilic layers better than dichlorvos. Comparing the physicochemical properties of these pesticides, it is predicted that the physicochemical differences of imidacloprid and dichlorvos may strongly affect the number of molecules entering the plant tissue. In other words, differences in the penetration rate of two pesticides changes the final biochemical status of the cucumber plants, which respond to pesticides.

Imidacloprid is a chloropyridinyl substituent and a systemic compound that can be transported within the xylem by acropetalic mobility (Sur and Stork 2003). It is well documented that the metabolism of imidacloprid in plants produces two important metabolites, 6-chloropyridinyl-3-carboxylic acid and 6-chloro-2-hydroxypyridinyl-3-carboxylic acid. Both compounds are salicylic acid (SA) mimic (Ford et al. 2010). In other words, imidacloprid and its metabolites are a structural analog of SA, which induce systemic acquired resistance inside the target plants (Szczepaniec et al. 2013). SA is one of the main phytohormones involved in both abiotic and biotic stress responses in plants (An and Mou 2011). It is well documented that SA as a signaling molecule plays a key role in both local defense reactions and the induction of systemic resistance (Khan et al. 2015). SA can be directly or indirectly involved in signaling pathways as well as interplays with ROS stressed plants (Raja et al. 2017). It maybe inferred that the remarkable responses of cucumber plants to imidacloprid pesticide may originate from SA action induced by imidacloprid. Thus, according to the importance of the SA pathway in host plants makes it necessary to conduct further investigations on endogenous SA content before and after treatments in cucumber plants.

Pesticide formulation may also affect volatilization, penetration uptake, and distribution into the plant cuticle (Lichiheb et al. 2016) and might be another reason for the different responses of cucumber plants to the pesticides used in this study. In addition, it has been reported that differences in adjuvants and auxiliary compounds used in the pesticide formulations can affect pesticide behavior inside plants (Gauvrit and Dufour 1990). Thus, it can be concluded that imidacloprid (suspension concentrate) and dichlorvos (emulsifiable concentrate) with different formulation types induce physiological pathways at different time scales in treated plants.

Conclusions

Results of this study show that imidacloprid and dichlorvos, which are used for cucumber plant protection against tobacco whiteflies, have profound effects on the host plant's physiology at the recommended rates. Host plants can seemingly overcome pesticide stress by improving their defense mechanisms, such as the antioxidative system, which may need to be consider as a problem if plants also encounter

other stresses, which can lead to increased ROS production since the antioxidative systems may already be at capacity to cope with ROS induced by the pesticides. On the other hand, it should be mentioned that some of the secondary metabolites, such as phenolic compounds, that are elevated in treated plants, have a direct role in plant–insect interaction. Increased concentrations of these metabolites can lead to changes of plant–insect interactions in favor of the host plants. Thus, plant–pesticide interactions are worth considering in the context of insect pest control. Different responses of the same plant to different pesticides maybe related to their physicochemical properties of the pesticide, which influences their effects in plants. Since plant secondary metabolism can dramatically change in response to stressors, including pesticides, more work on pesticide effects on plant metabolism should be conducted. The effects of pesticides on plant biochemical pathways may alter tolerance mechanisms of crops to herbivorous insect damage. Our results indicate that more work needs to be done to determine the economic thresholds for pesticides in crop protection.

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Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

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